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Direct Detection of the BRAF^{V600E} mutation in total RNA extracted from biopsies of melanoma patients

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Abstract

For the first time we show results of a clinical study with nanomechanical microcantilevers in a liquid environment to investigate malignant melanoma. Malignant melanoma comprises 3-4% of all malignant skin tumors nevertheless it is responsible for 60% of all fatal outcomes related to skin neoplasia. The number of malignant melanoma cases has increased 5 to 10 fold in the last 50 years. Especially in the later stages the disease is difficult to treat successfully and survival rates are low. Recently progress has been made and inhibitors to protein kinases, like BRAF, that have an influence on cell growth, were approved for treatment. The BRAF inhibitor targets a specific mutation in the protein called BRAF^{V600E}. The mutation is involved in 50% of all malignant melanoma cases. An important task is to identify the patients who have this mutation and therefore can profit from the treatment. The work presented here shows a method to detect the mutation in total RNA from biopsies and does not rely on amplification or labeling.

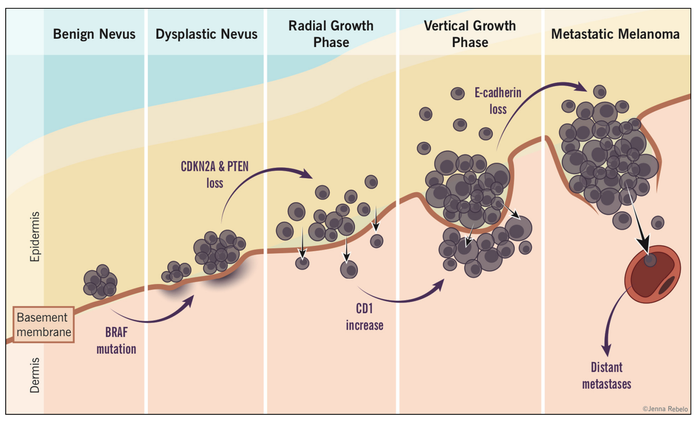

Fig. 1. Melanoma tumour progression, based on the Clark model (McMaster Pathophysiology Review).

Method

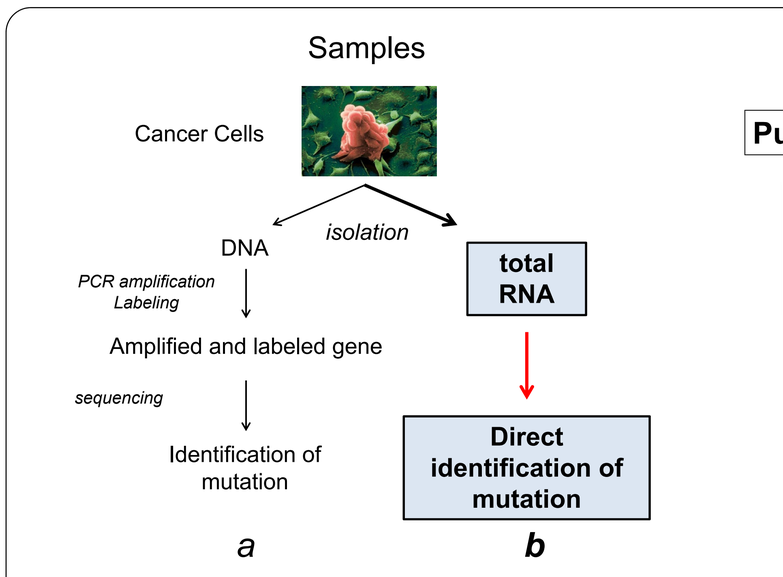

Fig. 2. *a* represents the standard procedure. ***b*** shows the new method which avoids amplification/labeling.

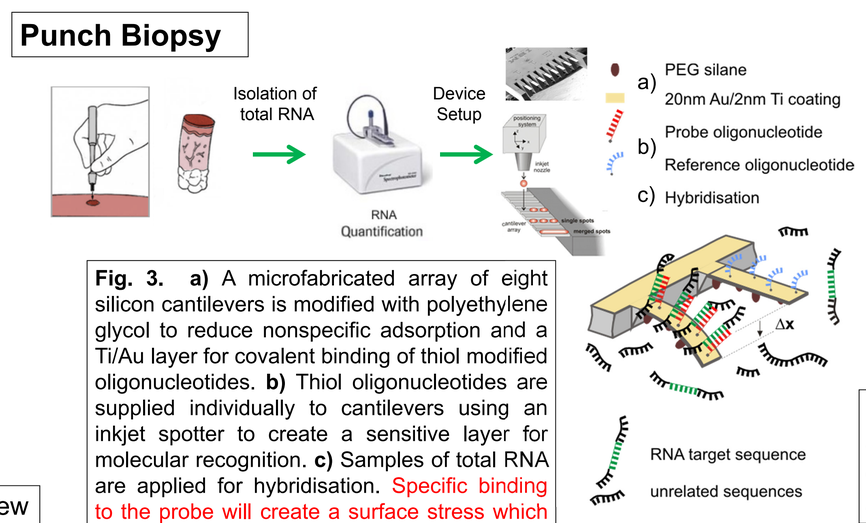

Fig. 3. **a)** A microfabricated array of eight silicon cantilevers is modified with polyethylene glycol to reduce nonspecific adsorption and a Ti/Au layer for covalent binding of thiol modified oligonucleotides. **b)** Thiol oligonucleotides are supplied individually to cantilevers using an inkjet spotter to create a sensitive layer for molecular recognition. **c)** Samples of total RNA are applied for hybridisation. Specific binding to the probe will create a surface stress which in turn results in the bending of the cantilevers.

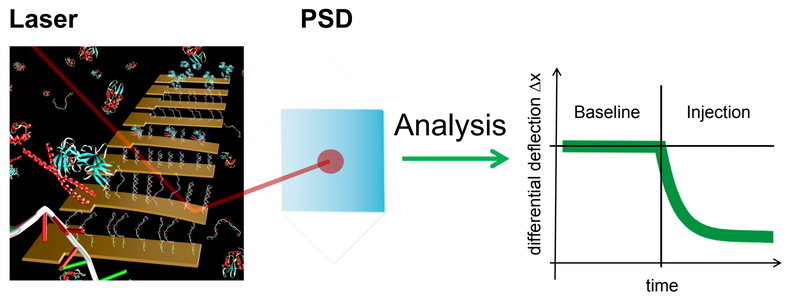

Fig. 4. An array of eight vertical cavity surface emitting lasers (VCSELs) and a position sensitive detector (PSD) are used for optical beam deflection measurements of the bending of each cantilever separately in a time-multiplexed way. The cantilever array is mounted in a liquid cell.

Results

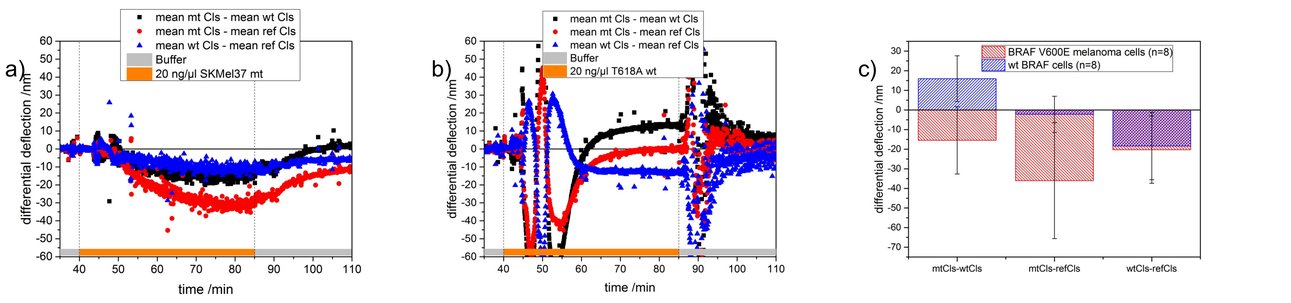

Fig. 5. a) Shows cantilever responses to binding of total RNA isolated from a BRAF^{V600E} positive cell line (SKMel37 mt). The red curve shows a strong response indicating the expression of the BRAF^{V600E} gene. This cell line has an additional complication as it also expresses the wild type gene, as seen with the blue response. b) on the contrary shows the response to a BRAF^{V600E} negative wild type cell line (T618A wt). As this cell line doesn't express the mutant BRAF^{V600E} gene we don't see a response in the red curve and only a strong blue signal, indicating the expression of the wild type gene. c) represents the summary of a total of 16 experiments. We can clearly distinguish the SkMel37 and the T618A cell lines.

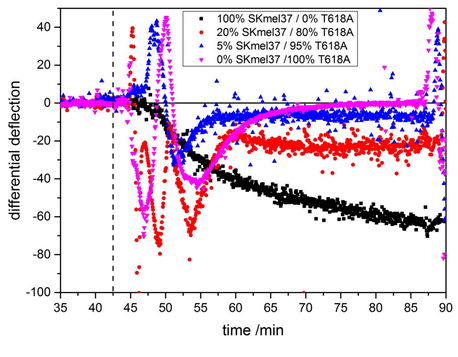

Fig. 6. Total RNA from mutant SKMel37 cells was diluted in total RNA from wild type T618 cells at different ratios. The lowest concentration we were able to identify was 5% SKMel37 in a sample.

Conclusions

Number	Amount of tot. RNA in µg	Lab Nr.	RNA conc. in ng/ul	Status	Evaluation
01.02 Form.	9.4	1b	188	mt	mt
01.03 Frozen	48.45	2a	969	wt	wt
01.04 Form.	78.15	2b	1563	wt	wt
01.05 Frozen	11.7	3a	234	wt	wt
01.06 Form.	417	3b	8340	wt	wt
01.07 Form.	626.95	4b	12539	mt	mt

Table 1 shows the summary of a clinical study with six samples of different origin, BRAF^{V600E} positive (mt) and negative (wt) patients. Samples were either frozen (Frozen) or formaldehyde (Form.) treated before total RNA was extracted resulting in a range of concentrations.

-The work shows that nanomechanical microcantilevers are able to identify mutations in complex samples ranging from cell lines to biopsies of patients.

-The technology is able to detect the mutation in a background of wild type sequences.

-We successfully concluded a first clinical study using nanomechanical sensors in a liquid environment. We plan to extend the study further to improve the statistical significance of our method.